SI Appendix

Ultrasmall Silica Nanoparticles Directly Ligate the T Cell Receptor Complex.

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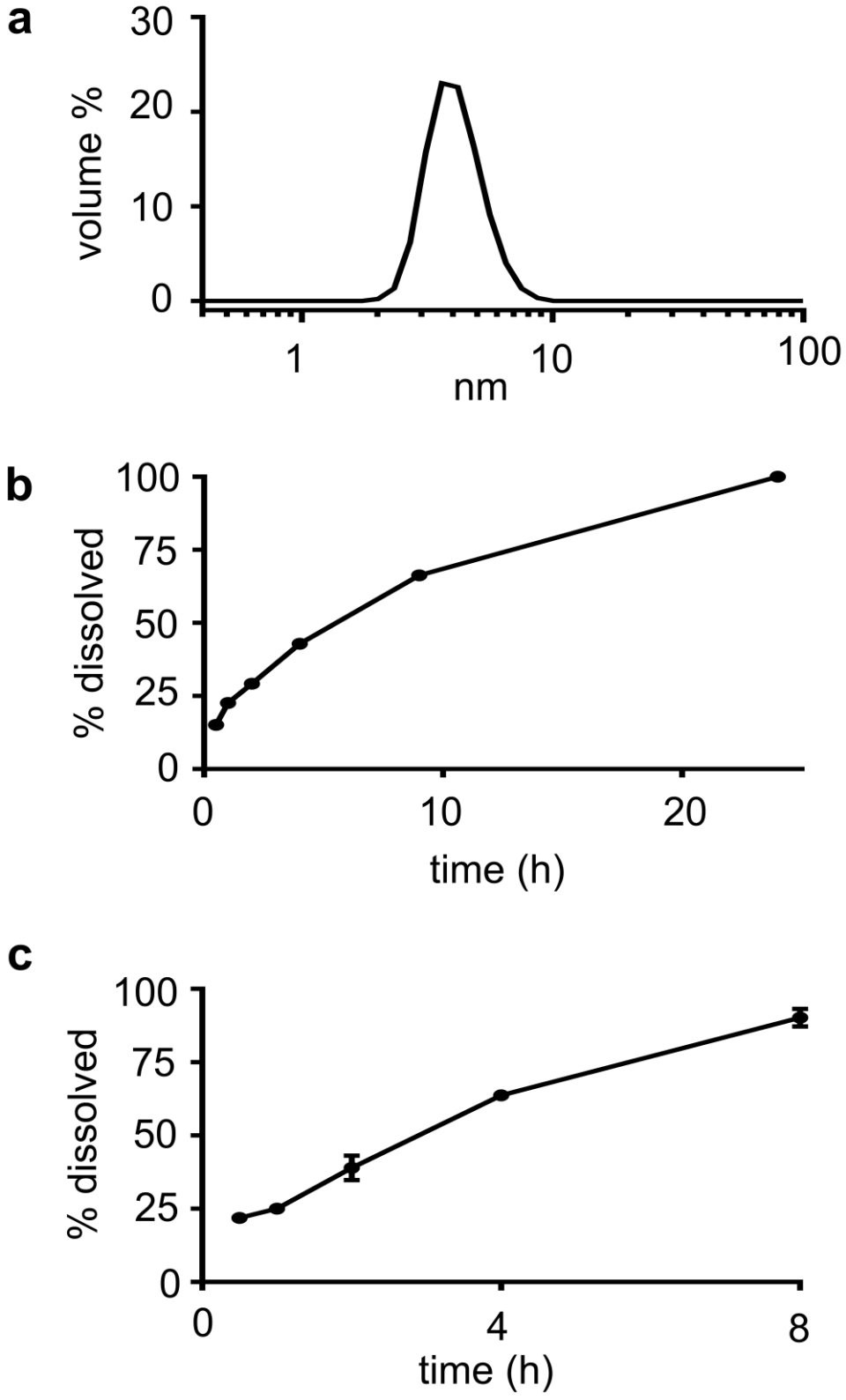
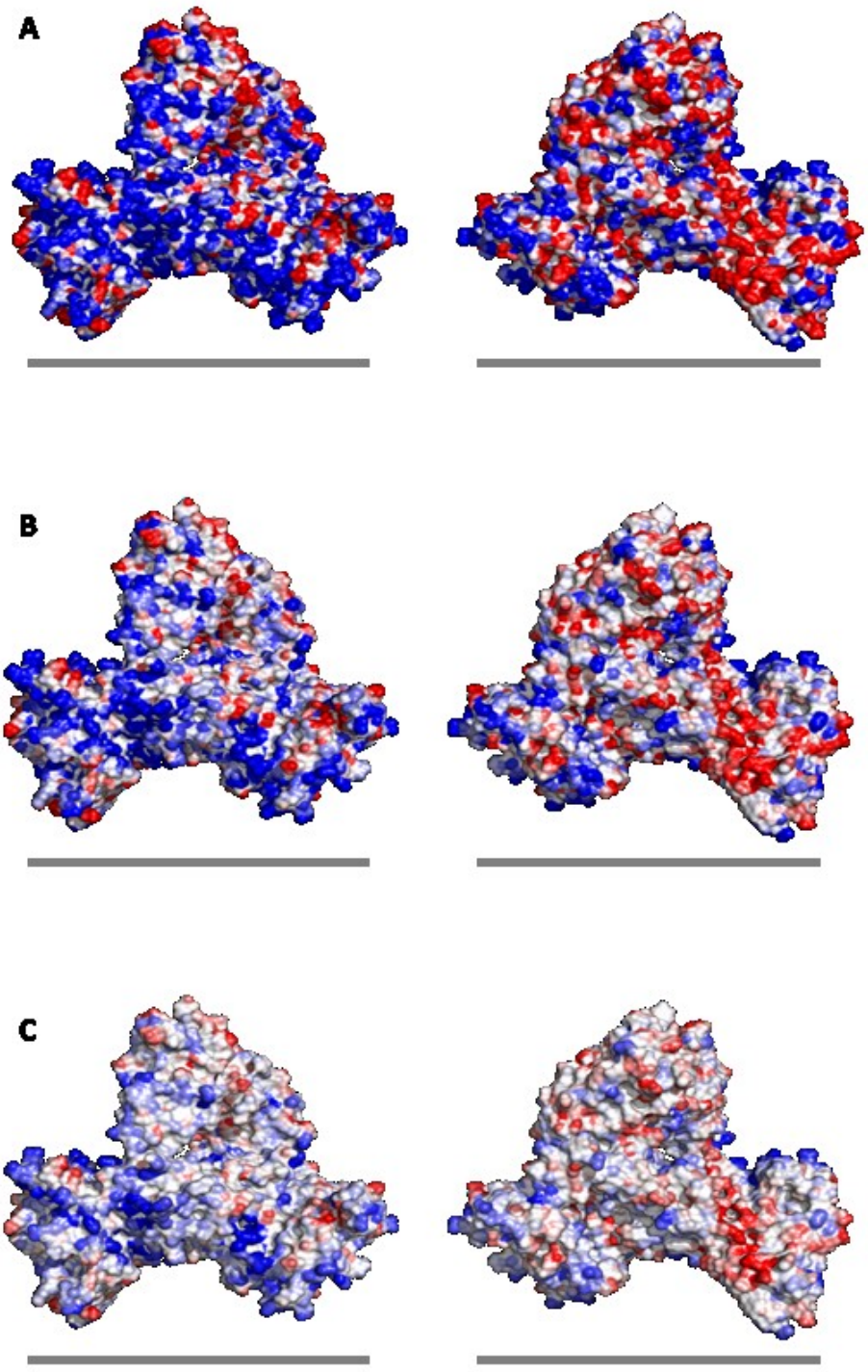
SI Appendix Figure S1. Characteristics of 3.6 nm USSN used in these investigations.

a. The size distribution of the USSN stock dispersion. **b.** Dissolution of 1000 μM USSN upon dilution in HEPES buffer (pH 7.0-7.3) at room temperature. **c.** Dissolution of 800 μM USSN upon dilution in RPMI media containing 10% fetal bovine serum at 37 °C.



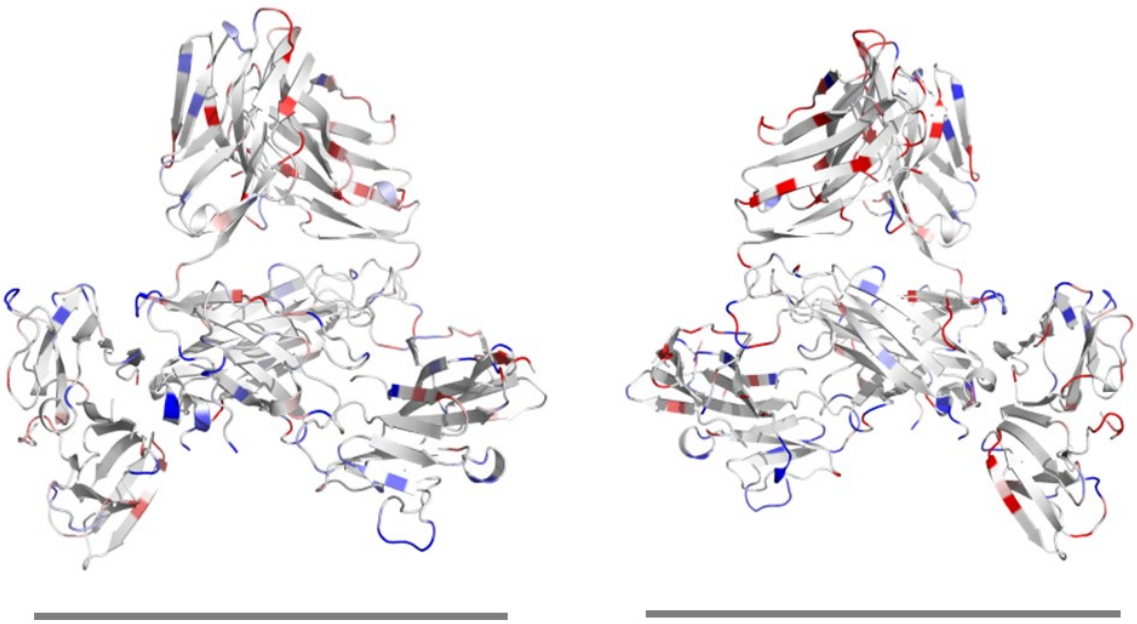
SI Appendix Figure S2. Electrostatic potential of modelled human TCR:CD3 complex.

Molecular surface representations of electrostatic potential were calculated using the Bluues algorithm and are shown over a range of ±2 (**A**), ± 5 (**B**) and ± 10 (**C**) kJ/mol q. The left and right sides of each panel represent front and back views of the receptor complex and are related by a 180° rotation around the vertical axis. Electropositive surfaces are blue and electronegative surfaces are red. Approximate location of the cell membrane is shown by a grey line. The image in **A** is that shown in main text Figure 2b and is included for comparative purposes.



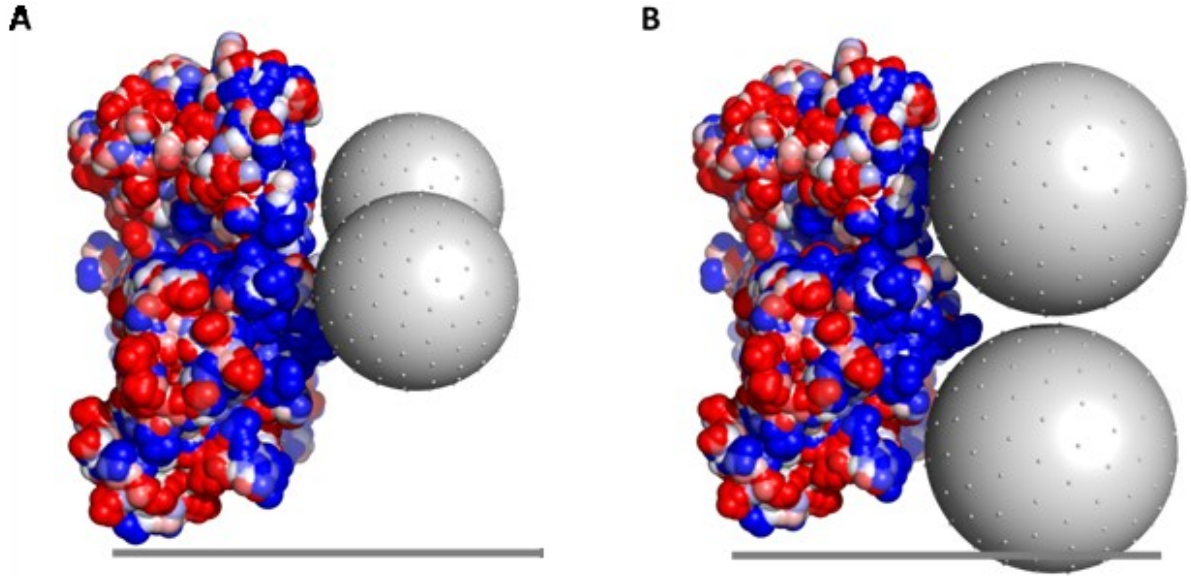
SI Appendix Figure S3. Ribbon representations of the modelled human TCR:CD3 complex.

The positions of the most electropositive (blue) and electronegative (red) amino acids on the human TCR:CD3 complex as calculated by Bluues. The left and right sides of each panel represent front and back views of the receptor complex and are related by a 180° rotation around the vertical axis. The grey line represents the approximate position of the cell membrane.



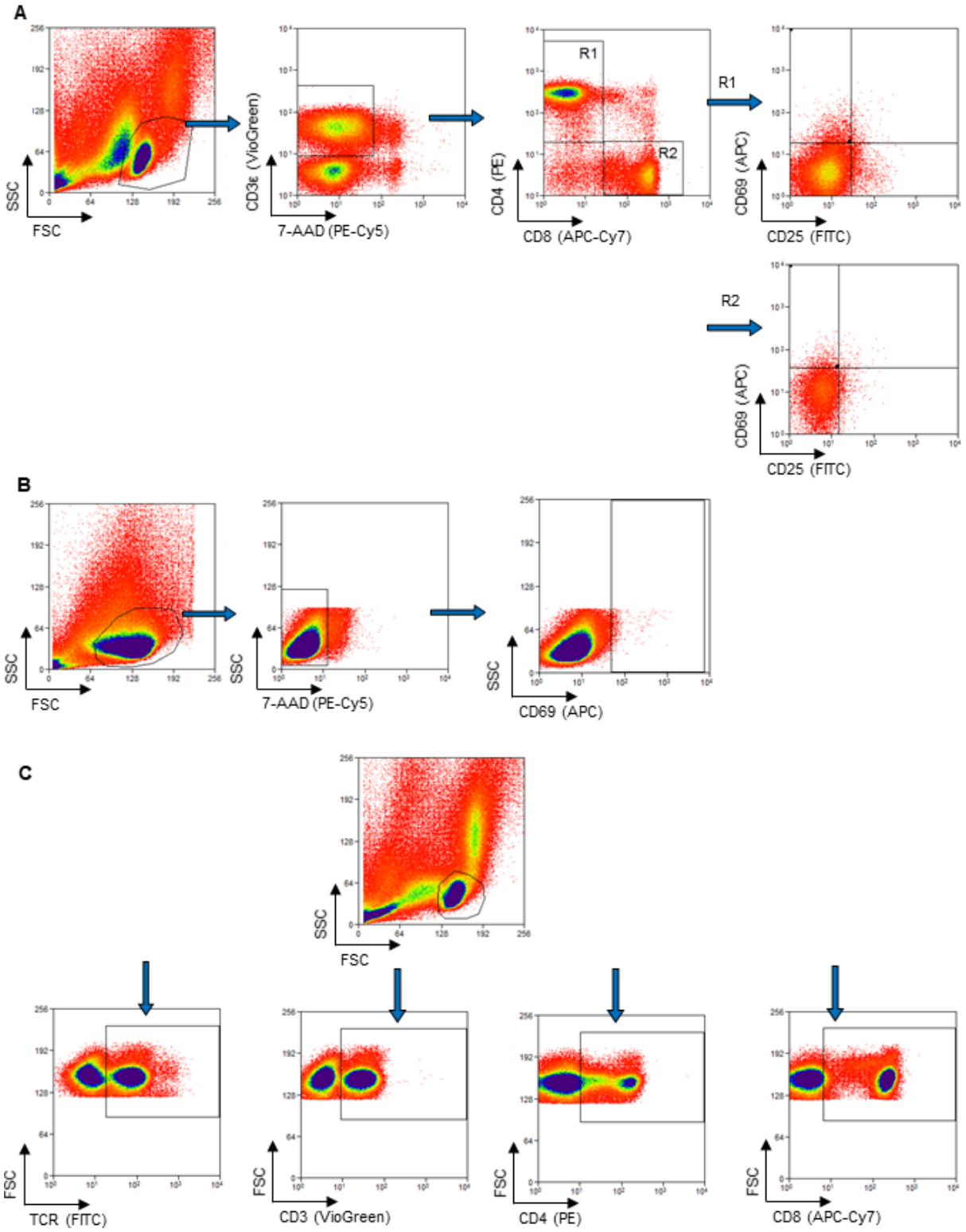
SI Appendix Figure S4. Side view representation of the solvent accessible surface of the human TCR:CD3 complex with potential locations of (A) 3.6 nm and (B) 5.0 nm USSN particles.

The USSN particles are visualised as grey spheres and the grey line provides an approximate position for the cell membrane. The TCR:CD3 complex is coloured by electrostatic potential across a range of -2 to +2 kJ/mol q. Electropositive surfaces are coloured blue and electronegative surfaces are coloured red.



Si Appendix Figure S5. Gating strategies used in the data analyses.

A. Gating strategy used to assess CD69 expression on CD4 and CD8 T cells in PBMC cultures. **B.** Gating strategy used to assess CD69 expression on Karpas-299 cells. **C.** Gating strategy used in the CD3ε and αβTCR competitive binding assay. **D.** Gating strategy used to assess CD25 and CD69 expression on γδ T cells in PBMC cultures. **E.** Gating strategy used in the γδ TCR competitive binding assay. **F.** Gating strategy used to assess CD4 and CD8 T cell proliferation expression in PBMC cultures.



Si Appendix Figure S5 continued.